EARLY EMBRYOGENY OF REBOULIA HEMISPHAERICA

(WITH FORTY-SEVEN FIGURES)

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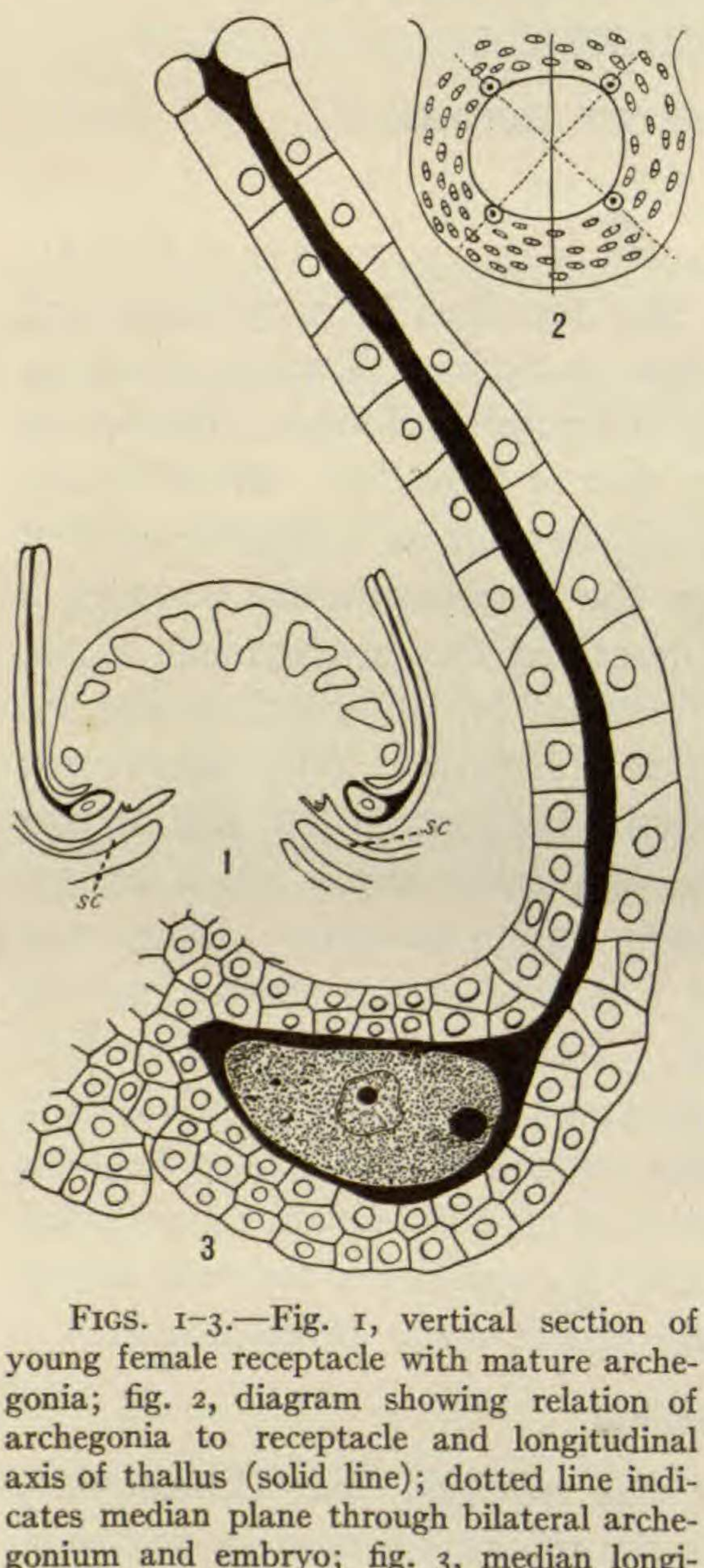
Our knowledge of the development of the sporophyte of *Reboulia* dates back to HOFMEISTER (11), who described its early stages and its rapid growth as it approaches maturity. KIENITZ-GERLOFF (13), who studied the embryogeny of a number of forms, claimed the *Reboulia* embryo to be similar to that of *Grimaldia*, certain stages of which he described in some detail, but not including the earliest stages. LEITGEB'S (14) work on the Marchantiaceae included a study of *Reboulia*, of which he described the development of the sporophyte in a general way. CAVERS' (3) observations also included the early and late sporophyte of *Reboulia*. The more recent work on the embryo has been done by WOODBURN (18) and HAUPT (10), the former dealing with the very early stages, the latter describing the development from beginning to maturity. These two recent accounts differ somewhat from the earlier studies, and in certain features from one another. A study of the writer's collections of material has yielded certain results which may be of interest in comparison with these accounts, especially where they bear on their differences, and in the addition of certain facts not mentioned by them. Altogether it is clear that the embryo of *Reboulia* shows considerable variation in the early phases of its development.

Material

The material for this study was collected near Huntingdon, Pennsylvania, the greater bulk of the embryos figured having been secured from collections made from the early part of October to the latter part of November, 1919. None of the material collected in September shows embryos so far as it has been examined. The early winter condition (figs. 46, 47) was secured from a collection made December 23, 1920. The material was killed in 25 per cent chrome-acetic acid, and for the most part stained by the iron-alum haemotoxylin method. As DURAND (7) found in *Marchantia*, the embryo stains much lighter than the surrounding calyptra, and it was found that dipping the slides for a short time in an alcoholic solution of Lichtgrün served to bring out clearly the delicate cell walls. This clouds the cytoplasm of the cells to a slight extent, and is not to be recommended when the details of cell structure are to be studied.



FIGS. 1–3.—Fig. 1, vertical section of young female receptacle with mature archegonia; fig. 2, diagram showing relation of archegonia to receptacle and longitudinal axis of thallus (solid line); dotted line indicates median plane through bilateral archegonium and embryo; fig. 3, median longitudinal section of mature archegonium; dark area about embryo represents space between embryo and calyptra; fig. 3, ×400.

Archegonium

The writer has found nothing in the development of the archegonium differing from the account given by HAUPT (9). It first appears when the female receptacle is yet quite small, and when the sex organ is mature the receptacle is still a low conical structure (fig. 1), surrounded by a large number of sterile scales (fig. 2). The venter of the archegonium is inclined somewhat below the horizontal, the neck curving upward more or less to a perpendicular position among the scales. HAUPT (9) regards these scales as probably protective in function. They also probably serve in holding a film of water about the archegonia, functioning much as do the paraphyses about the sex organs of mosses, resulting in conditions which increase the probabilities of fertilization.

Usually there are four archegonia on each receptacle, one close behind the apical cell of each of the four growing points of the receptacle. Rarely three, occasionally five or six such growing points and archegonia may occur. In the typical condition the archegonia are so situated on the receptacle that a median section through the entire archegonium can be secured only by vertical sections cut on a plane at an angle of 45° to the long axis of the thallus (fig. 2). Owing to their curvature, both the archegonium and the early embryo are bilateral and not radial, and a strictly median section can pass through but one plane. With but few exceptions all the embryos figured in this account are from sections along this plane.

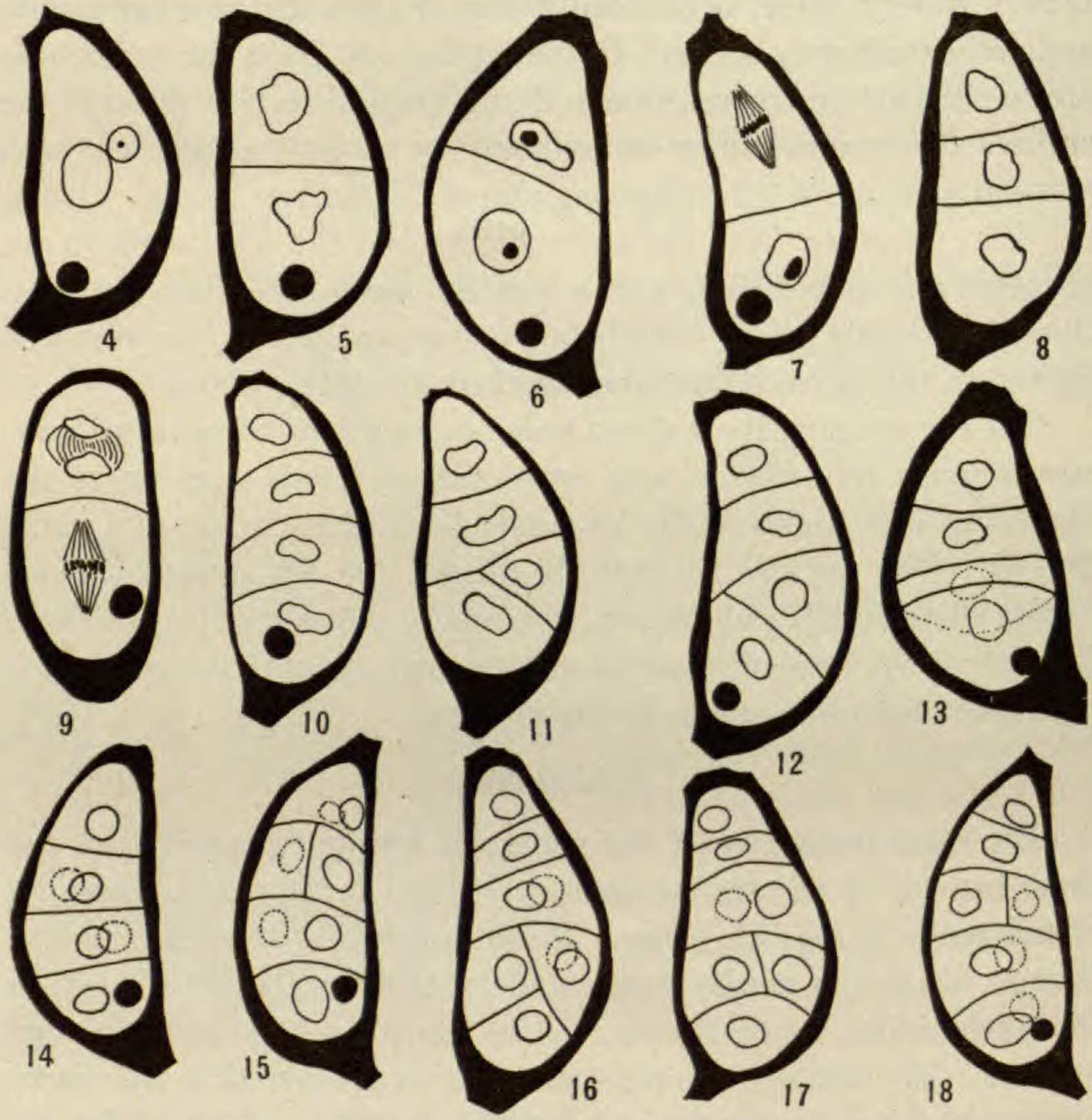
The egg at maturity is about twice as long as its transverse diameter, bluntly rounded at both ends, slightly more tapering at the hypobasal end, and with its long axis describing the arc of a circle (fig. 3). The nucleus is centrally placed, the egg cytoplasm uniformly distributed, and containing plastids and oil globules. There is usually a very conspicuous oil globule near the anterior end, which persists even in late stages of the embryo.

Fertilization

The close proximity of the male and female receptacles on the same branch of the thallus usually insures fertilization, although occasionally it fails to occur. WOODBURN calls attention to the change which the motile sperm undergoes from the time it leaves the antheridium until its nucleus is ready to fuse with the egg nucleus. The writer found a number of cases in which the sperm nucleus had penetrated the egg cytoplasm and lay close to the egg nucleus (fig. 4). At this time the egg nucleus has about twice the diameter of the sperm nucleus, whose more compacted chromatin results in a denser staining body. No attempt was made to study the nuclear changes involved in fertilization. Apparently the fusion nucleus passes into the resting stage before division of the egg takes place.

Embryo

FIRST DIVISION.—Without any considerable enlargement after fertilization, the egg divides by a transverse wall, usually perpendicular to the long axis, and giving nearly equal epibasal and hypobasal cells (fig. 5). This agrees with the accounts for *Reboulia* as given by CAVERS (3), WOODBURN (18), and HAUPT (10), and with



FIGS. 4–18.—Fig. 4, egg with male and female nuclei, black circle representing oil drop; fig. 5, first wall transverse; fig. 6, first wall oblique; fig. 7, mitosis in hypobasal cell; fig. 8, embryo of three cells; fig. 9, mitosis in both epibasal and hypobasal cells; fig. 10, typical filamentous embryo of four cells; figs. 11–13, epibasal cell divided by oblique wall; fig. 14, vertical division of two middle cells of row, basal and apical cells undivided as yet; fig. 15, apical cell not yet divided by vertical wall; fig. 16, epibasal cell divided by oblique wall, vertical division in middle of embryo, basal cell of row of four probably transversely divided; figs. 17, 18, two cells at base probably resulting from transverse division of basal cell; ×430.

such forms as *Targionia* (CAMPBELL 1, O'KEEFE 16), *Plagiochasma* (STARR 17), *Conocephalum* (CAVERS 2), *Riccia* at times (GARBER 8), and practically all the Jungermanniales which have been examined, among which are *Sphaerocarpus* and *Geothallus* (CAMPBELL 1), *Aneura* (LEITGEB 14, CLAPP 5), *Fossombronia* (HUMPHREY 12), *Pellia* (KIENITZ-GERLOFF 13), and *Symphyogyna* (MCCORMICK 15), and is in contrast with forms in which the first wall is more or less oblique, as occurs in *Riccia* (KIENITZ-GERLOFF 13, CAMPBELL 1, GARBER 8), *Marchantia* (DURAND 7), and *Preissia* (KIENITZ-GERLOFF 13).

HOFMEISTER thought the *Reboulia* egg divided first by a strongly inclined wall. LEITGEB claimed the first wall to be generally oblique, occasionally perpendicular, to the long axis. HAUPT (10) states that the first division is "always accompanied by a transverse wall." WOODBURN'S statement is not so positive, and one of his figures shows the first wall slightly inclined. The writer found several cases in which the first wall was more or less oblique, sometimes with the epibasal cell the larger of the two (fig. 6).

FILAMENTOUS EMBRYO.—The published accounts differ considerably as to the behavior following the first division. HOFMEISTER described the growth as due to an apical cell with two cutting faces, the epibasal cell dividing repeatedly by alternately inclined walls, resulting in a slender embryo of two rows of cells. KIENITZ-GERLOFF was not convinced by HOFMEISTER'S account, but concluded from analogy with *Grimaldia* that in *Reboulia* an octant is formed by vertical walls perpendicular to the first transverse wall. LEITGEB claimed quadrant formation by walls perpendicular to the first, the apical and basal cells of the quadrant being the larger, since the first wall is usually oblique, and both the epibasal and hypobasal cells are divided unequally. CAVERS (3) also claimed an octant by the formation of perpendicular walls, and regarded the epibasal half of the octant as giving rise to the capsule, the hypobasal to the foot and stalk. The studies by WOODBURN, HAUPT, and the writer do not agree with these earlier statements. In most cases the second and third divisions are parallel to the first, resulting in a filament of four cells, with the walls between them more or less parallel to one another.

WOODBURN and HAUPT both claim the second division to be in the epibasal cell, resulting in a row of three cells. WOODBURN says the third division may be in either the apical or the middle of these three cells; HAUPT states that it is in the apical cell. According to both workers the first division of the hypobasal cell does not take place until after the formation of the row of four cells, and then usually by a vertical wall. HAUPT says that occasionally this vertical wall formation takes place before the third transverse division, that is, when the embryo consists of but three cells. Neither WOODBURN nor HAUPT show mitotic figures definitely proving this sequence of division. The sequence of these early divisions may hold a definite relation to the later differentiation of the sporophyte into foot, stalk, and capsule regions. The writer's preparations show that transverse division may take place in both hypobasal and epibasal cells (fig. 9), and that the division of the hypobasal cell precedes that of the epibasal cell (fig. 7). The division of the hypobasal cell may be completed before the epibasal cell begins to divide, resulting in a three-celled filament (fig. 8). Such embryos are probably quite rare, however, the writer having found but a single case. It is more probable that the division of the epibasal cell is generally initiated before that of the hypobasal cell is complete (fig. 9), and the four-celled embryo results with the completion of the two division processes (fig. 10). The writer concludes, therefore, that at this stage the embryo consists typically of a row of four cells with parallel walls, as a result of the transverse division of both hypobasal and epibasal cells, the division of the former preceding slightly that of the latter. Not a single case was found suggestive of the quadrant, as claimed by CAVERS, who probably based his interpretation on later stages without having observed these early ones, as he shows no figures of early embryo development.

Variations from the typical situation are of interest. For example, the transverse walls are often more or less curved, with the concave side toward the apex, the curvature often being especially pronounced in case of the apical cell (figs. 10, 17); or the division of the epibasal cell may be by an oblique wall (figs. 11–13) whose inclination to the perpendicular may show considerable variation. Such a division of the epibasal cell is more likely to occur when the first division has been an oblique one (figs. 11, 12), although the inclination of this wall may be independent of the first wall (figs. 13, 16). WOODBURN figures several embryos showing oblique walls in the epibasal portion. HAUPT thinks oblique walls do not occur at this stage or later. It seems to the writer that the evidence for the occasional occurrence of oblique walls is conclusive.

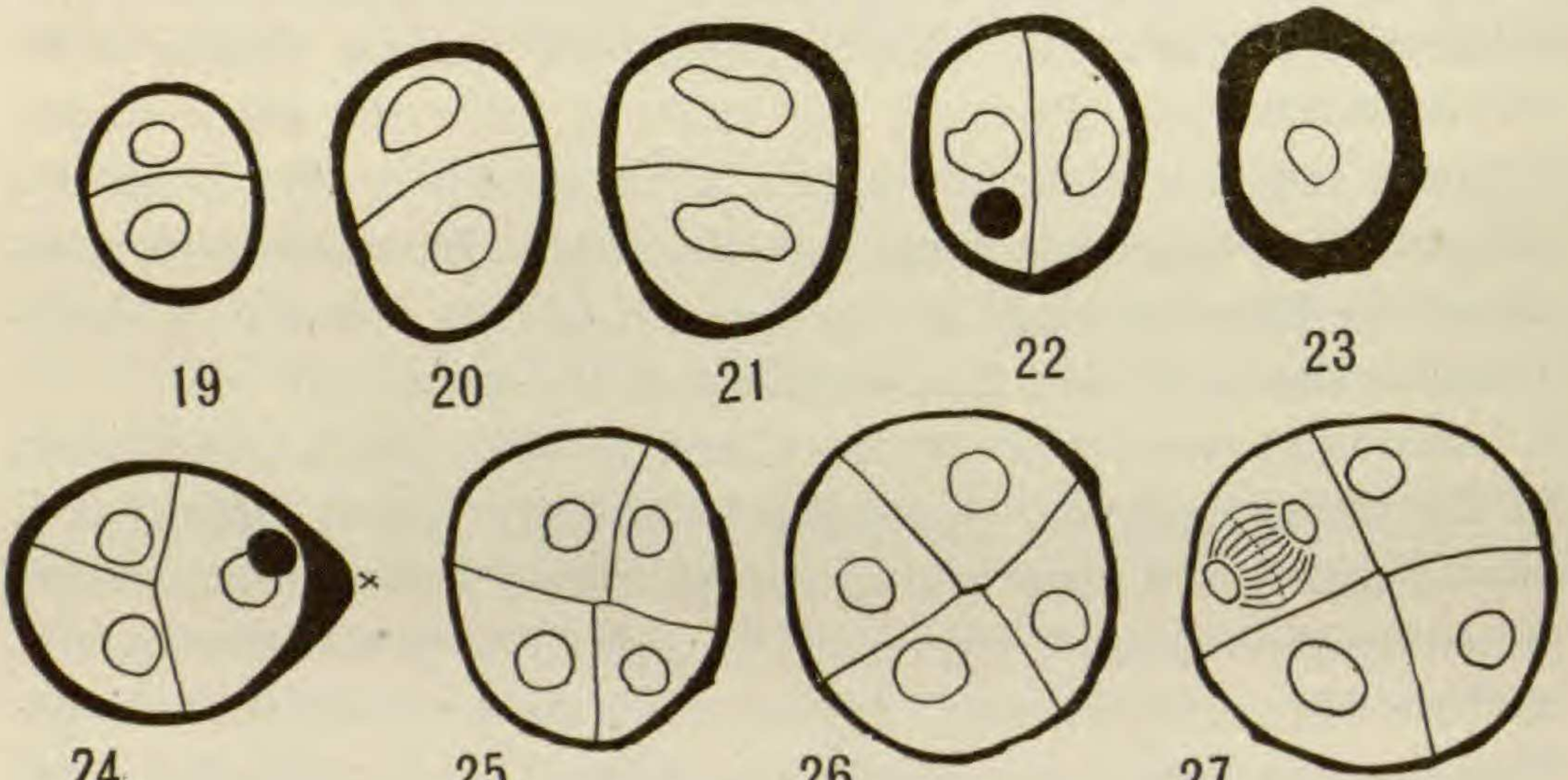
The occurrence of a filamentous embryo in *Reboulia* agrees with the embryo described for *Plagiochasma* (STARR 17), *Targionia* (O'KEEFE 16), *Geothallus*, and *Sphaerocarpus* (CAMPBELL), as well as practically all the Jungermanniales. Quadrant formation by walls vertical to the first wall occurs in *Riccia* (CAMPBELL 1), in *Marchantia*, as given by a number of writers, DURAND's account being the most complete, *Conocephalum* (CAVERS 2), and *Fimbriaria californica* (CAMPBELL). KIENITZ-GERLOFF made a similar claim for *Grimaldia* and *Preissia*, but did not have the early stages. GARBER found occasionally a row of three cells in *Riccia natans*, although the quadrant form was the rule. In the following account the innermost cell of the filament of four cells will be designated as the basal cell, the outermost as the apical cell.

VERTICAL WALL FORMATION.—Vertical walls now begin to form in the young embryo. According to HAUPT, "these vertical divisions begin at the lower end of the embryo, a feature which is also noted by WOODBURN's figures." This probably is the general rule, and is evidenced by HAUPT's figures, which show mitoses in the hypobasal portion before occurring in the epibasal portion, the basal cell evidently dividing first. It is quite common to find embryos of this stage with the apical cell yet undivided (figs. 14, 15). This cell also soon divides, either by a vertical wall or otherwise, as described later. A series of cross-sections of an embryo at this stage shows that the vertical walls do not usually lie in the same plane, but are inclined to one another at various angles (figs. 19–23). These vertical walls are usually perpendicular to the transverse ones, which, if obliquely inclined, usually result in oblique vertical walls, and the embryo may, in surface view of this and later stages, appear spiral. Occasionally some of the vertical walls are oblique to the transverse wall, even in the middle of the filament (figs. 17, 28).

The first vertical walls are soon followed by a second series, usually at right angles to the first, typically dividing each segment into four cells (figs. 25, 26). These divisions may be more or less simultaneous (fig. 36), although not ordinarily so even in the same segment.

Should the apical cell also divide by the two series of vertical walls, the apex of the embryo will consist of four octohedral cells (fig. 33). This is the situation as described by HAUPT. LEITGEB describes a similar condition in *Blasia*. Owing to the curvature of the embryo two of these will be nearer the neck of the archegonium than the other two.

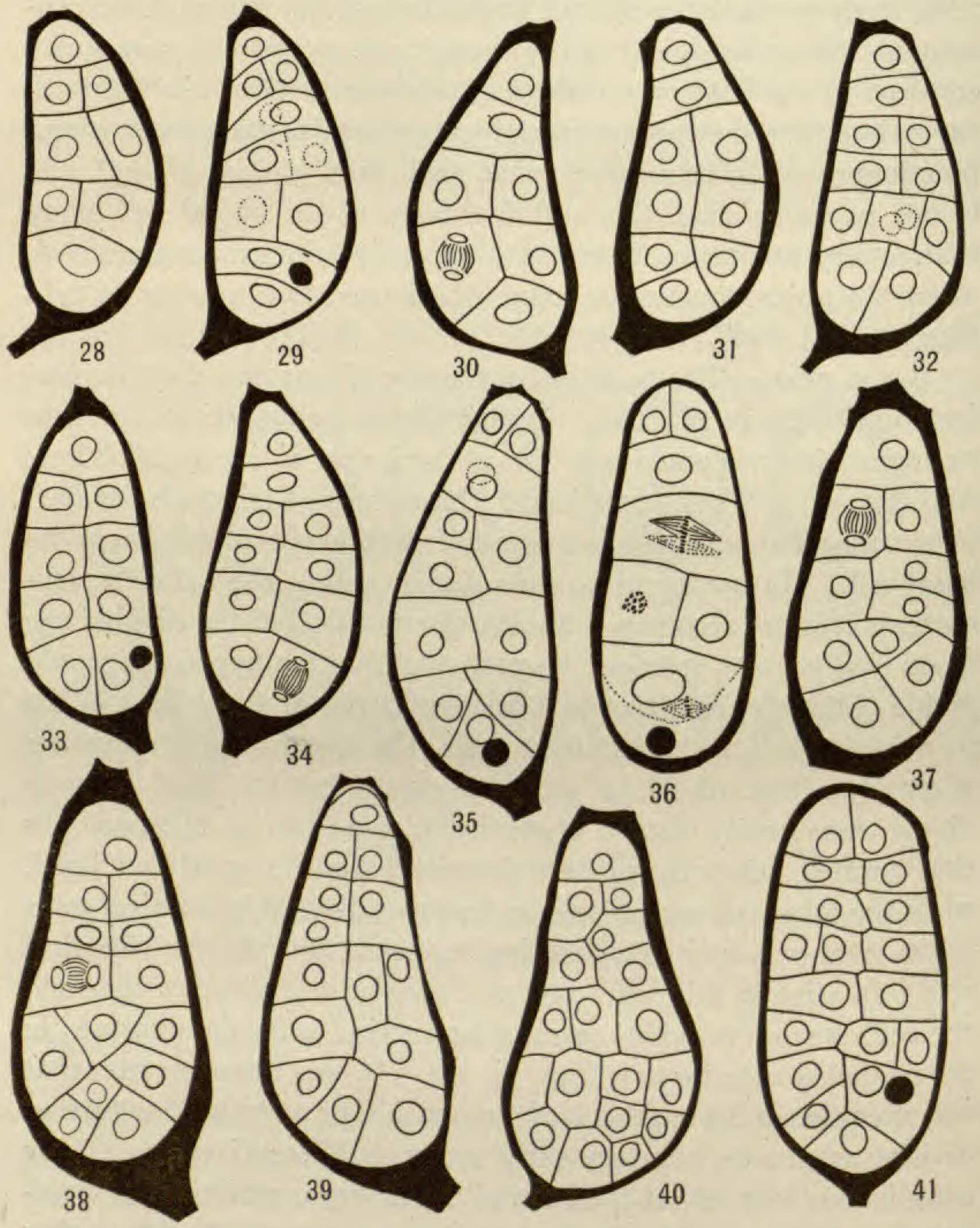
APICAL CELL.—The apical cell of the row of four may divide vertically, or, as certain embryos suggest, it may divide again transversely (fig. 29) before vertical division takes place, although in



FIGS. 19–27.—Figs. 19–23, series of cross-sections through embryo, showing inclination of vertical walls to one another in successive segments; fig. 19 is basal segment; fig. 23, apex; fig. 24, transverse section of apical segment of embryo, *x* is neck of archegonium; figs. 25–27, transverse section of embryo with segments in quadrants; compare fig. 27 with fig. 42, noting position of walls; ×570.

the absence of a mitosis one cannot be absolutely positive on this point. It may also divide by an oblique wall, whether its basal wall is oblique or transverse (figs. 31, 32, 34, 35). WOODBURN shows a similar situation, to which HAUPT takes exception, claiming that in his investigation "a truly median section has never revealed the presence of a triangular apical cell." LEITGEB found that in *Blasia* the apical cell may divide by oblique walls, and he figures several embryos with a triangular apical cell. The writer's observations of *Reboulia* confirm WOODBURN's statement. In fact, owing to the curvature of the embryo, a truly median section is the one most likely to show the oblique inclination of the wall and the consequent triangular apical cell, although one would not necessarily err even in the interpretation of an oblique section. It seems to the writer that there is no room for doubt as to the presence and functioning of a triangular apical cell (figs. 34–39, 41, 45). It is not probable that this cell functions as an apical cell more than a very few times, being soon "lost" in the growing embryo, where the apical function becomes distributed to a number of cells (figs. 40, 46).

BASAL CELL.—The basal cell of the row of four also does not perform uniformly in all cases. It may divide by a vertical wall into two approximately equal cells (fig. 36), or it may divide by an oblique wall (fig. 35). WOODBURN found "basal cells of triangular shape" to occur, probably arising as a result of oblique wall formation in the basal cell. HAUPT found no case of this, and in the writer's preparations it is not common. Should the first wall of the divided egg be an oblique one, it might be probable that the hypobasal would divide obliquely, resulting in a triangular cell at the base. While no mitosis was found as a direct proof, the appearance of a number of embryos (figs. 16–18, 30–32, 34) suggests that the basal cell may divide transversely instead of vertically, the basal of the two cells thus formed behaving as here described for the basal cell itself, while the other cell sooner or later becomes divided by vertical walls in the same way as its neighboring segment (figs. 28, 29). Vertical wall formation in this cell would probably be delayed for a time and the cell remain undivided, even after vertical walls have formed in the segments anterior to it (figs. 34, 36). It seems to the writer that very frequently the basal cell of the row of four undergoes no further division whatever, but very early becomes differentiated as a large conspicuous foot cell at the base of the embryo, retaining its hemispherical shape, very early showing denser contents than the other cells of the embryo, and becoming coated on its free margin by a heavy thickening (figs. 45–47). This cell often remains quite distinct, even in late embryos, and may clearly be recognized both in sections (fig. 47) and in surface views of dissected embryos. Should the basal cell have divided transversely (as already suggested) the basal of the two cells formed may remain undivided. WOODBURN



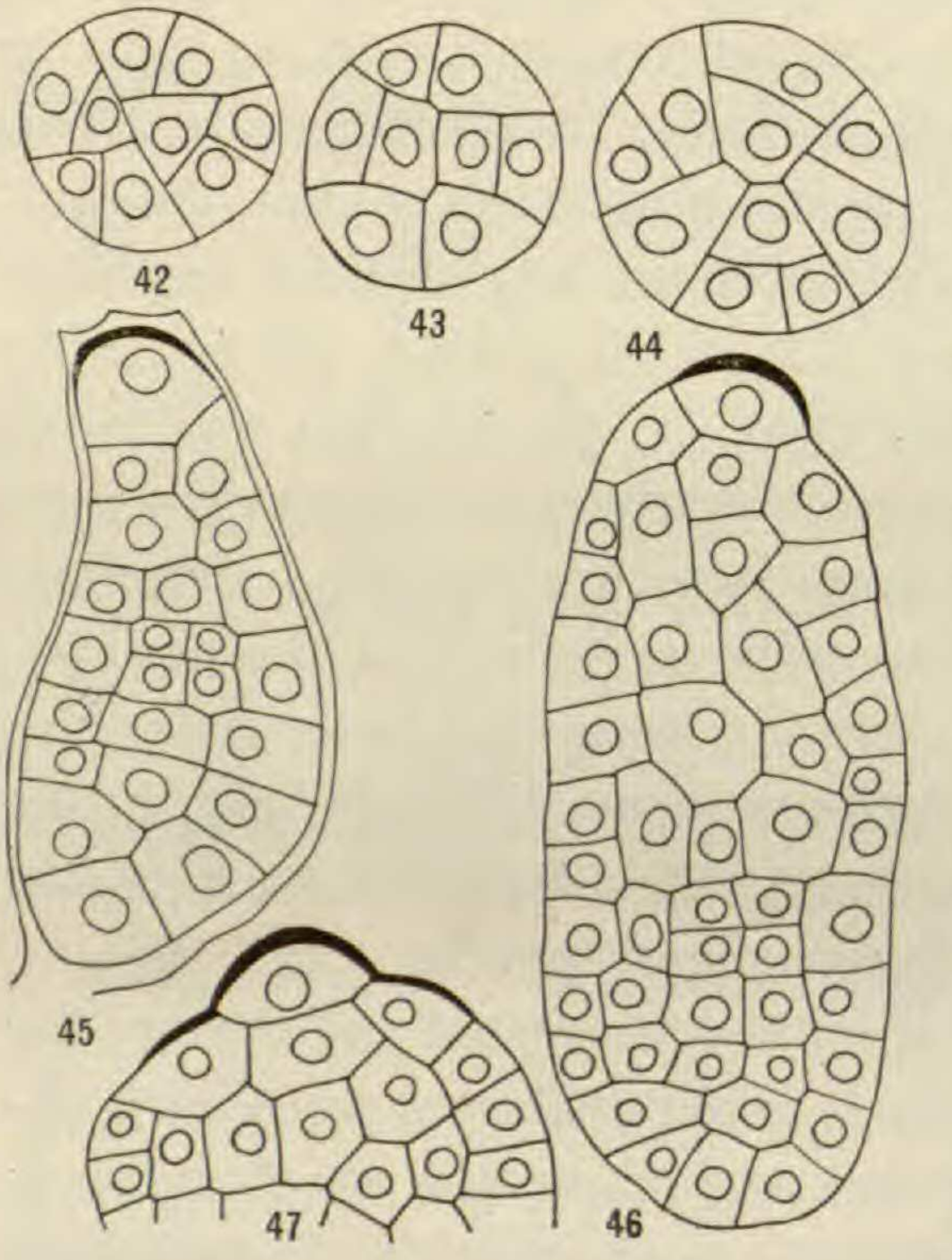
FIGS. 28–41.—Fig. 28, embryo with segment divided by oblique wall; fig. 29, apical cell (of row of four), probably divided transversely; figs. 30–41, embryos showing varying features, figs. 36, 37, and 41 cut at plane perpendicular to median longitudinal plane; in fig. 36 division is going on at several different regions of embryo; dotted line at apex represents oblique wall separating deeper cell (in process of division) from superficial cell with oil drop and undivided nucleus; figs. 34, 35, 37–39 show functional triangular apical cell; $\times 430$.

cites a case where the basal cell has divided into a small group of irregular cells, probably also a very rare feature.

It is evident that the basal cell of the filamentous embryo undergoes but few, and in some cases no further divisions, and therefore makes a relatively small contribution to the tissue of the sporophyte, which therefore is built up almost entirely from the three anterior cells of the filament of four cells.

LATER GROWTH AND EMBRYO DIFFERENTIATION.—Along with or following the formation of vertical walls, transverse divisions in some or all of the segments result in additional tiers of cells, the divisions occurring in different planes without any definite sequence (figs. 36–41). At first the ventral side of the embryo will probably show the greater number of cells (figs. 35, 39, 40), but as growth continues the dorsal side also grows rapidly, and the embryo soon becomes a radial instead of a bilateral structure (fig. 46). Periclinal walls now form, especially in the epibasal portion of the embryo, without any definite sequence, forming inner and outer cells (figs. 38–44). These first periclinal walls are most likely to form in the capsule-forming region of the embryo, which becomes considerably broader than the more slender hypobasal portion (figs. 40–45). This portion, however, soon broadens out somewhat and reaches the winter condition (fig. 46). While it is impossible to trace back absolutely the origin of the different regions of the sporophyte, it seems most probable from the writer's study that the first division of the egg determines the capsule region as distinguished from the foot and stalk region, the epibasal cell giving rise to the capsule, therefore, the hypobasal to the stalk and foot, with the bulk of both foot and stalk derived from the anterior half or three-fourths of the original hypobasal cell. They are derived from the anterior half if the basal cell (of the row of four) does not undergo tiansverse division, and from the anterior three-fourths if the basal cell should divide transversely. Both WOODBURN and HAUPT regard the foot as derived from the hypobasal cell, the stalk and capsule from the epibasal cell; the capsule being formed from the two anterior cells of the three derived from the epibasal cell, according to HAUPT. The sequence of the early divisions and the behavior of the hypobasal portion of the embryo would seem to warrant the writer's interpretation.

While it may be probable that the future sporogenous tissue is cut off from the capsule wall by the first periclinal walls which form in this portion of the embryo, it does not show differential staining until later, when the physiological differentiation becomes evident, as shown in the more massive capsule (fig. 46) of the winter condition. CAVERS (4) holds to the view that "the capsule wall in Marchantiales is not differentiated until a relatively late stage; that is, the separation of the archesporium is not determined by the first periclinal divisions in the young capsule." Further development takes place the succeeding spring, with the sporophyte reaching maturity, in this latitude from the middle of May to the middle of June. The writer has not made a careful study of sporogenesis, HAUPT'S paper giving an account of the features in detail.



FIGS. 42-47.—Figs. 42-44, transverse sections of embryos showing first periclinal walls; fig. 45, longitudinal section of young embryo late in November (note prominent basal cell); fig. 46, embryo in winter condition; ×350; fig. 47, base of embryo in winter condition (large basal cell quite conspicuous).

Calyptra and involucre

The calyptra grows apace as the embryo develops, becoming several layered and relatively somewhat massive. The longitudinal axis of both embryo and calyptra becomes more and more vertical, until finally it is practically perpendicular to the substratum, with the neck of the archegonium hanging downward. The calyptra incloses the sporophyte until spring, when the rapid growth of the latter breaks through the slower growing calyptra and becomes exposed, excepting where covered by the receptacle tissue, which has grown downward and formed an involucre about both calyptra and sporophyte, dorsally and laterally.

Discussion

The finding of filamentous embryos in an increasing number of Marchantiales makes it evident that the octant type of embryo is not necessarily the rule in this group, in contrast with the filamentous embryo of the Jungermanniales. This, together with the occurrence of oblique walls and even a triangular apical cell, tends to bring the Marchantiales and Jungermanniales closer together as regards their embryogeny, and in an occasional partial agreement with that characteristic of the Musci.

In a previous paper, the writer (6) referred to the plasticity of *Reboulia* as shown by the male reproductive structures. The variations found in the development of the embryo give additional support to that view.

In the differentiation of the capsule region from the foot and stalk, *Reboulia* is probably like that of most Marchantiales, in that the capsule is generally derived from the epibasal half of the egg. Even in *Reboulia* there is no absolute proof that the epibasal cell may not contribute in part to the stalk region, as is the case in the Jungermanniales. The behavior of the basal cell of the filament is suggestive of an approach to the situation in some of the Jungermanniales where the entire hypobasal cell is a mere appendage to the embryo.

Summary

1. The mature egg and early embryo are elongated, slightly curved, bilaterally symmetrical bodies.

2. Fertilization takes place in October, the development of the embryo beginning at once, the sporogenous tissue becoming differentiated by winter, the sporophyte maturing in May and June.

3. The early embryo shows considerable variation in its development, the chief features being: (1) the first division of the egg may be transverse or oblique; (2) transverse division of both hypobasal and epibasal cells results in a filamentous embryo of four cells;

(3) vertical wall formation occurs in these four cells, with the exception, commonly, of the basal cell; (4) oblique walls may occur in any part of the embryo, and are not uncommonly to be found in the apical region where they may form a triangular apical cell, functional in the cutting off of a few segments; (5) the foot and stalk are probably derived from the hypobasal cell, the epibasal cell giving rise to the capsule, although it may conceivably make some contribution to the stalk as well; (6) the basal cell of the row of four varies in its contribution to the tissue of the foot, at times apparently remaining undivided, in which case the remainder of the foot and the stalk is derived from its sister cell.

4. The variations in the early embryo support the view that *Reboulia* is a plastic form, and as such may occupy a genetic position among the Hepaticae.

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